Effects of F-strain *Mycoplasma gallisepticum* Inoculation at Twelve Weeks of Age on Digestive and Reproductive Organ Characteristics of Commercial Egg Laying Hens[1,2]

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ABSTRACT Experimental inoculation with the F-strain of *Mycoplasma gallisepticum* (FMG) between 8 and 18 wk of age is known to affect reproductive performance in commercial layers. Therefore, two trials were conducted to determine if changes in digestive and reproductive organ characteristics also occur in commercial laying hens infected with FMG at 12 wk of age. In Trial 1, liver weight, liver lipid and moisture contents, ovary weight, ovarian follicular hierarchy, and the weights, lengths, and histologies of the infundibulum, magnum, isthmus, uterus, and vagina were determined. In Trial 2, fatty liver hemorrhagic syndrome (FLHS) incidence and the weights, lengths, and histologies of the duodenum, jejunum, and ileum were determined in addition to the parameters examined in Trial 1. In both trials, the average number of mature (diameter ≥ 12 mm) ovarian follicles was lower in FMG-inoculated hens in comparison to controls. Also, magnum/oviduct (cm/cm) length was reduced in treated birds. In Trial 2, isthmus/BW and isthmus/oviduct (g/g) weight were decreased at 46 wk of age, and vagina/BW and vagina/oviduct (g/g) weight were decreased at both 20 and 36 wk of age due to FMG treatment. In Trial 2, FMG treatment resulted in a 50% increase in the number of FLHS birds. Furthermore, treatment caused a decrease at 20 wk of age and an increase at 44 wk of age in liver moisture content. However, the intestinal characteristics examined were not affected by FMG inoculation. Altered liver, ovarian, and reproductive organ characteristics were associated with FMG infection in commercial layers. More specifically, FMG inoculation at 12 wk resulted in a higher incidence of FLHS, ovarian follicular regression, and decreased isthmal and vaginal proportions of the reproductive tract. These data clearly demonstrate that alterations in performance and egg characteristics of layers inoculated with FMG at 12 wk of age are related to mutual functional disturbances in the liver, ovary, and oviduct without concomitant intestinal changes.

(*Key words*: layer, liver, *Mycoplasma gallisepticum*, reproductive tract, small intestine)

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INTRODUCTION

Mycoplasma gallisepticum (MG) is an infectious gram negative bacterium (Razin and Freundt, 1984), which infects nearly the entire flock, and tends to be more severe in young birds and during cold weather (Ley and Yoder, 1997). Layer hens infected with MG develop conjunctivitis and other air sac distresses (Soeripto et al., 1989; Nunoya et al., 1995). A detailed histological examination of MG-infected chicken air sacs was provided by Trampel and Fletcher (1981). In that report, increases in total volume and numbers of epithelial cells, heterophils, mononuclear cells, fibrin, blood vessels, and connective tissue components were found in the air sacs from chickens that had been inoculated with MG 21 d earlier. Also, it has been documented that MG can be cultured from tracheal, air sac, lung, and sinus exudates (Kleven and Yoder, 1989) and the choanal cleft/palatine fissure (Branton et al., 1984), as well as the brain (Chin et al., 1991). Along with affecting the respiratory apparatus, MG also affects the reproductive performance of commercial layers (Mohammed et al., 1987; Burnham et al., 2002). Branton et al. (1997, 2000) reported that egg production beginning at 22 wk of age and other egg characteristics in birds inoculated with the *F*-strain MG (FMG) at 10 wk of age were not

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Abbreviation Key: FH = follicular hierarchy; FLHS = fatty liver hemorrhagic syndrome; FMG = F-strain of *Mycoplasma gallisepticum*; HI = hemagglutination-inhibition; MS = *Mycoplasma synoviae*

different from controls. However, in a more recent investigation using the same birds as in this study being reported, Burnham et al. (2002) reported that egg production and other egg characteristics beginning at onset of lay (18 wk of age) in birds inoculated with FMG at 12 wk of age were different from uninoculated controls. Burnham et al. (2002) reported that initiation of lay was delayed and that weekly egg production after 42 wk and overall average weekly egg production were reduced in layer hens inoculated with FMG at 12 wk of age. Early inoculation or vaccination with FMG may help reduce losses in the performance of birds as a result of infection by field strains of MG (Luginbuhl et al., 1976; Yoder et al., 1984).

Mycoplasma gallisepticum can be vertically transmitted from a hen to her eggs (Glisson et al., 1984). Also, MG has been cultured from the oviduct (Carlson and Howell, 1967; Domermuth et al., 1967; Hitchner et al., 1980), liver, spleen, uterus, and vagina (Sahu and Olson, 1976) and cloaca (Amin and Jordan, 1979; MacOwan et al., 1983) of chickens. This information warrants investigation into the possible involvement of digestive and reproductive organ changes with those of performance in MG-infected hens. No literature is available concerning these organ system characteristics in MG-infected layers. Hepatic lipidosis, referred to as fatty liver syndrome, often precedes fatty liver hemorrhagic syndrome (FLHS), which has been associated with environmental heat stress in birds (Riddell, 1997). However, Branton et al. (2002) also reported that FLHS incidence was delayed in hens inoculated at 10 wk of age with *Mycoplasma gallinarum*. Because *Mycoplasma gallinarum* is nonpathogenic in avian species (Kleven, 1991), the affects of FMG, a pathogenic strain, on FLHS incidence was also investigated in this study.

This study was designed to characterize possible physiological changes, including digestive and reproductive organ characteristics, throughout a complete egg laying cycle in commercial layers vaccinated with FMG. Physiological changes may be associated with previously described alterations in performance. The organs examined included the liver, small intestine, ovary, and oviduct. Furthermore, the relative lengths and weights of the individual segments that comprise the small intestine and oviduct were examined.

MATERIALS AND METHODS

Pullet Housing and Management

In each of two trials, one thousand 1-d-old pullets of a single genetic strain were obtained from a commercial source that was monitored and certified free for MG and *M. synoviae* (MS) (National Poultry Improvement Plan and Auxiliary Provisions, 1995). Chicks were vaccinated at 10 d of age for infectious bursal disease via the drinking water. At 12 d and again at 4 wk of age, chicks were also vaccinated for Newcastle Disease and infectious bronchitis by the same route. At 5 wk of age, ten randomly selected pullets were bled from the left cutanea ulnea wing vein and tested for antibodies to MG and MS using both the serum plate agglutination and the hemagglutination-inhibition (HI) tests (Yoder, 1975). At the same time, swabs were collected from the choanal cleft (Branton et al., 1984) and placed into tubes containing Frey's broth medium (Frey et al., 1968) supplemented with an additional 0.15 mg thallium acetate and 10^6 IU penicillin g/mL. Tubes were incubated at 37 C for 30 d or until a phenol red indicator reaction occurred in the media. A sample from those that changed color was then inoculated onto Frey's-based (Papageorgiou medium) agar and incubated at 37 C. Colonies with morphology suggestive of *Mycoplasma* species were examined by an agar plate fluorescent antibody method (Baas and Jasper, 1972) that used direct labeling of colonies stained with anti-FMG polyclonal antibodies produced in rabbits and labeled with fluorescein isothiocyanate (Kleven, 1981).

Until the pullets were 12 wk of age, they were placed on clean dry litter in a 5.5×6.1 m section of a conventional house resulting in an initial flock density of 0.034 m^2/bird. A daily artificial lighting schedule followed a 13 L:11 D cycle. One 75-W incandescent light bulb was used to illuminate each 8.4 m^2 of floor space, providing a calculated intensity at bird level of 35.5 lux. Feed and water were provided ad libitum in each trial. Ingredient percentages and dietary analyses of the basal starter and grower diets used in both trials were reported by Burnham et al. (2002). All diets were formulated to meet or exceed National Research Council (1994) specifications. No medication was administered during the interval of either trial.

At 12 wk of age, 11 pullets were randomly selected and placed in each of 8 (Trial 1; total of 88 pullets) or 16 (Trial 2; total of 176 pullets) negative pressure fiberglass biological isolation units (1.16 m^2). The units were housed in a previously described poultry disease isolation facility (Branton and Simmons, 1992). Ingredient percentages and dietary analyses of the basal developer and prelay diets used in both trials were reported by Burnham et al. (2002).

Layer Housing and Management

Hen numbers were reduced to 10 per unit at point-of-lay (18 wk of age), so that bird density was 0.116 m^2/bird for the duration of each trial. In each trial, half of the total number of isolation units contained FMG-free control birds, whereas the other half contained FMG-inoculated birds. There were four replicate units per treatment in Trial 1, and eight replicate units per treatment in Trial 2. Beginning at 18 wk of age, the artificial lighting schedule was increased 15 min/d until a cycle of 16 h 15 min L:7 h 45 min D was achieved. Chickens were maintained on that schedule through the remainder of the experiments. Ingredient percentages and dietary analyses of the layer diets used in both trials were reported by Burnham et al. (2002). In both trials at 26 and 54 wk of age, quadruplicate feed samples per lot of mixed feed were analyzed for moisture, ash, CP, crude fat, and crude fiber. All determined analyses were performed according to the methods of the Association of Official Analytical

Chemists (1980) and averaged for each of the two trials at each time period. Available protein and lysine percentages in the layer diet were adjusted according to the percentage of feed consumed per bird every 28 d until trial termination (54 wk in Trial 1 and 60 wk in Trial 2).

FMG Inoculation

In each trial, pullets treated with FMG were inoculated via eye drop in the right eye at 12 wk of age with 0.04 mL of a 24-h broth culture of high-passage FMG (99th passage above the unknown passage level) provided by S. H. Kleven.[4] Inoculum titers were 5.0×10^6 and 1.0×10^5 cfu/mL in Trials 1 and 2, respectively. Similarly, pullets designated as controls were sham-inoculated via eye drop in the right eye at 12 wk of age with 0.04 mL of sterile Frey's broth medium.

Mycoplasma Detection

In each trial at 20 wk, and again at 54 wk in Trial 1 and 58 wk of age in Trial 2, one randomly selected hen from each of four FMG-free control and FMG-treated isolation units was bled and swabbed. Each of these samples were tested for the presence of *Mycoplasma* species as previously described for pullets.

Data Collection

Trial 1 was terminated at 54 wk and Trial 2 at 60 wk of bird age. At those times, five birds from each of four control and four treated units in Trial 1 and four birds from each of six control and six treated units in Trial 2 were randomly selected and euthanized by cervical dislocation and their organs removed. Similarly, in Trial 2, two randomly selected birds from each of two control and two treated units were euthanized and their organs removed at 20, 36, 44, 46, and 48 wk of age. In both trials, organ analyses included liver weight, lipid, and moisture content, ovary weight and follicular hierarchy, and the weights, lengths, and histologies of the oviduct, infundibulum, magnum, isthmus, uterus, and vagina. In Trial 2, incidence of FLHS and the weights, lengths, and histologies of the duodenum, jejunum, and ileum were also examined in addition to the above mentioned parameters. Intestinal and oviductal segment weights were calculated as percentages of total body and organ weight, and segment lengths were calculated as percentages of total organ length.

Mature Follicle Quantitation

In both trials, the entire ovary was removed and the number of mature (diameter ≥ 12 mm) yellow ovarian follicles was recorded for each bird. Sturkie and Mueller (1976) reported that a mature bird's ovary contains four to six large yolk-filled follicles (20 to 40 mm in diameter) within a follicular hierarchy (F1 = largest follicle, F2 = second largest follicle, etc.). However, in the current study preovulatory follicles in mature birds greater than or equal to 12 mm in diameter were recorded. This range was chosen to include all large yolk fluid-filled follicles that are destined to be ovulated. A caliper was used to measure follicle diameter. Based on the number of mature ovarian follicles present in each bird, a categorical number from zero to seven was assigned. A number of zero indicated that a bird had no mature follicles present. A maximum of seven mature follicles were recorded. Average number of mature follicles and the percentage of birds having zero, one, two, three, four, five, six, or seven mature follicles in each replicate unit were analyzed.

Liver Moisture and Lipid Analysis

For analysis of liver moisture content, fresh liver samples (2 g) were dried according to the procedure of Peebles et al. (1999) in a commercial oven.[5] Liver moisture content was calculated as the difference between the wet and dry weights of the sample and was expressed as a percentage of wet sample weight. For analysis of liver lipid content, lipid was extracted from fresh liver samples (3 g) according to the procedure previously described by Bligh and Dryer (1959) and as modified by Latour et al. (1998). Liver lipid content was expressed as a percentage of total fresh liver sample weight.

Histopathologic Examination

Upon termination of each trial, one tissue sample from the ovary, infundibulum, magnum, isthmus, uterus, and vagina was harvested from one hen in each of four units in both control and treatment groups. Tissue samples were placed in 10% buffered neutral formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. Each tissue sample was observed and scored for the presence or absence of lymphoid and heterophil infiltrates as described by Branton et al. (2000). Treatment assignments were unknown to the evaluator.

Statistical Analysis

A completely randomized experimental design was utilized. All data were subjected to a one-way analysis in Trial 1 and a repeated measures analysis in Trial 2. Individual sample data within each replicate unit were averaged prior to analysis. Least squares means were compared in the event of significant global effects (Steel and Torrie, 1980; Petersen, 1985; Freund and Wilson, 1997). All data were analyzed using the MIXED Procedure of SAS (1996). Statements of significance were based on $P \leq 0.05$ unless otherwise stated.

RESULTS

In both trials, all initial mycoplasmal cultures as well as SPA and HI test results obtained from 5-wk-old pullets

[4]University of Georgia, Athens, GA.
[5]Model EL20, General Electric Co., Chicago Heights, IL.

were negative for MG and MS. Control serum samples obtained at 20 wk of age in each trial and also at 54 wk (Trial 1) and 58 wk (Trial 2) were serum plate agglutination and HI negative for MG, while the same tests were positive for MG in the FMG-inoculated hens. Hens were considered FMG-free when they exhibited no detectable HI titers. All FMG-inoculated hens had HI titers ≥ 1:80. Similarly, fluorescent antibody culture results for swabs obtained at 20 wk of age in each trial and also at 54 wk (Trial 1) and 58 wk (Trial 2) were negative for *Mycoplasma* species growth for four out of four FMG-free hens tested, while growth was evident for four out of four FMG-inoculated hens tested. At necropsy, all digestive and reproductive tracts appeared normal through gross observation. Interestingly, one FMG-inoculated hen possessed functional left and right reproductive tracts. No differences were observed through histopathologic lesion scoring between treatments for any of the tissues sampled.

When expressed as percentages of BW, main effects due to layer age were observed for liver ($P \leq 0.0001$), ovary ($P \leq 0.0001$), oviduct ($P \leq 0.004$), infundibulum ($P \leq 0.009$), magnum ($P \leq 0.03$), uterus ($P \leq 0.0001$), small intestine ($P \leq 0.0001$), duodenum ($P \leq 0.0001$), jejunum ($P \leq 0.0001$), and ileum ($P \leq 0.004$) weights in Trial 2. In that same trial, liver lipid content changed ($P \leq 0.005$) with hen age. Furthermore, infundibulum weight ($P \leq 0.05$) as a percentage of oviduct weight, absolute lengths of the small intestine ($P \leq 0.006$) and oviduct ($P \leq 0.0001$), and lengths of the infundibulum ($P \leq 0.0002$), magnum ($P \leq 0.003$), isthmus ($P \leq 0.0001$), uterus ($P \leq 0.0001$), and vagina ($P \leq 0.0001$) as percentages of total oviduct length were affected by layer age. In general, oviduct and small intestine weights and lengths from birds in Trial 2 increased normally over the entire experimental period. Oviduct and small intestine weights reached approximately 52.06 and 18.74 g, respectively, and their subsequent lengths reached approximately 69.32 and 111.50 cm, respectively, at 60 wk of age in Trial 2. Also, in that same trial, percentage liver lipid content was 8.96% at 20 wk and 10.22% at 60 wk of age.

In Trials 1 and 2, there was a main effect ($P \leq 0.05$) due to FMG inoculation on numbers of mature ovarian follicles. In both trials, the average number of mature follicles in FMG-inoculated birds was lower compared to those in controls. In Trial 1, FMG-free hens averaged 5.33 mature follicles, while FMG-inoculated hens averaged 5.00 mature follicles (SEM = 0.124). Likewise, in Trial 2, FMG-free hens averaged 5.33 mature follicles, while FMG-inoculated hens averaged 4.63 mature follicles (SEM = 0.207).

In Trial 1, there was a main effect due to FMG inoculation for the percentage of birds having five ($P \leq 0.009$) or six ($P \leq 0.03$) mature ovarian follicles (Table 1). The percentage of FMG-inoculated birds having five follicles was higher, while the percentage of FMG-inoculated birds having six follicles was lower in comparison to those in sham-inoculated control birds. Inoculation with FMG, therefore, resulted in an overall reduction in ovarian follicle size as demonstrated by a drop in the number of mature follicles (from six to five) in FMG-inoculated birds. There was a main effect ($P \leq 0.02$) due to FMG inoculation on magnum length as a percentage of oviduct length in Trial 1. Magnum length as a percentage of oviduct length in FMG-inoculated birds was decreased in comparison to that in controls. Magnum length as a percentage of oviduct length was 45.11 in control hens and 43.17 in FMG-inoculated hens (SEM = 0.387).

In Trial 2, there was a main effect ($P \leq 0.0009$) due to FMG inoculation for incidence of FLHS. The percentage of FLHS in FMG-inoculated birds was higher (48.77%) compared to that in control birds. There was a 7.43% incidence of FLHS in control hens and a 56.20% level of FLHS incidence in FMG-inoculated hens (SEM = 9.707).

In Trial 2, there was an age by FMG treatment interaction ($P \leq 0.04$) for percentage liver moisture (Table 2). Inoculation with FMG resulted in a decrease in liver mois-

TABLE 1. Percentage of F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated Single Comb White Leghorn laying hens having zero, one, two, three, four, five, six, or seven mature (diameter ≥ 12 mm) ovarian follicles for both trials across sampling period between 20 and 60 wk

	Trial 1[3]		Trial 2[4]	
Number of follicles	FMG free (%)	FMG inoculated (%)	FMG free (%)	FMG inoculated (%)
o	0.0	0.0	0.0	4.2
1	0.0	0.0	4.2	4.2
2	0.0	0.0	0.0	0.0
3	0.0	0.0	5.1	9.7
4	6.7	5.0	1.6	21.0
5	53.3[b,1]	90.0[a]	54.2	31.3
6	40.0[a,2]	5.0[b]	25.8	16.7
7	0.0	0.0	9.0	12.5

[a,b]Means within trial and number of follicles among treatment groups with no common superscript differ significantly ($P \leq 0.05$).

[1]Based on pooled estimate of variance SEM = 6.22.

[2]Based on pooled estimate of variance SEM = 7.99.

[3]n = 40.

[4]n = 88.

TABLE 2. Percentage liver moisture in F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated Single Comb White Leghorn laying hens at 20, 36, 44, 46, 48, and 60 wk of age in Trial 2

Age (wk)	FMG free[1] (%)	FMG inoculated[1] (%)
20	29.3[b]	34.8[a]
36	26.5	26.1
44	32.2[a]	26.3[b]
46	31.7	33.6
48	27.0	28.6
60	26.8[2]	27.2[2]

[a,b]Means within week of age among treatment groups with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 4 duplicate samples, and based on pooled estimate of variance SEM = 1.58 for each treatment at Weeks 20, 36, 44, 46, and 48.

[2]n = 24 duplicate samples, and based on pooled estimate of variance SEM = 0.65 for each treatment at Week 60.

ture content at 20 wk followed by an increase at 44 wk of age. In Trial 2, there were age by FMG treatment interactions for isthmus weight as a percentage of BW ($P \leq 0.04$) and as a percentage of oviduct weight ($P \leq 0.006$) (Table 3), and for vaginal weight as a percentage of BW ($P \leq 0.01$) and as a percentage of oviduct weight ($P \leq 0.05$) (Table 4). Isthmus/BW and isthmus/oviduct (g/g) weights were decreased at 46 wk of age, and vagina/BW and vagina/oviduct (g/g) weights were decreased at both 20 and 36 wk of age due to FMG.

DISCUSSION

Many investigators have reported the impacts of both field and vaccine strain infections of MG in birds (Van der Heide, 1977; Gentry, 1978; Carpenter et al., 1981; Lin and Kleven, 1982; Hildebrand et al., 1983; Glisson et al., 1984; Yoder et al., 1984; Branton and Deaton, 1985; Khan et al., 1986; Mohammed et al., 1987; Stadelman, 1988; Kleven et al., 1990; Burnham et al., 2002). In each of these reports, whether the investigator used field or vaccine strains of MG, layer hen egg production was altered or reduced. *Mycoplasma gallisepticum* may colonize various regions of the female reproductive tract and disrupt egg formation. Earlier reports indicated that MG may be cultured from the oviduct (Carlson and Howell, 1967; Domermuth et al., 1967; Hitchner et al., 1980), liver, uterus, vagina (Sahu and Olson, 1976), and cloaca (Amin and Jordan, 1979; MacOwan et al., 1983) of chickens. Later, Glisson et al. (1984) also reported that hens may transmit MG to their eggs via this same reproductive pathway. These reports indicate that MG may have the unique ability to colonize and impair certain reproductive processes in commercial birds. At the beginning and end of both trials in this study, serum plate agglutination tests from swabs and sera and HI sera tests, along with the fluorescent antibody tests verified systemic infections in FMG-inoculated birds. Conversely, sham-inoculated birds remained FMG-free throughout each trial. These data also showed that FMG inoculation at 12 wk resulted in a higher incidence of FLHS, ovarian follicular regression, and decreased isthmal and vaginal proportions of the reproductive tract.

The ovaries of egg producing birds usually contain four to six large yolk-filled follicles (2 to 4 cm in diameter), accompanied by a greater number of smaller (2 to 10 mm in diameter) follicles and numerous tiny white follicles (Sturkie and Mueller, 1976; Johnson, 2000). Delayed onset of lay in treated birds (Burnham et al., 2002) may be associated with a delay in ovarian follicular development during prepeak egg production. As indicated in each of the current trials, fewer mature (diameter ≥ 12 mm) follicles existed in FMG-inoculated hens than in controls. Ovarian regression may be related to retarded production (liver), transport (blood), and uptake (ovary) of yolk particles. Atrophic ovarian follicles may be a consequence of FMG colonization in specific ovarian cell groups. It has been proposed by Williams and Sharp (1978) and Palmer and Bahr (1992) that decreases in egg production (% hen/d) with hen age is in part caused by both an increase in the incidence of atresia and a reduction in the number of follicles that reach the final phase of rapid growth. Consequently, fewer follicles receive a greater proportion of yolk, resulting in larger sized eggs. The influence of FMG on ovarian tissue function may, therefore, imitate those of the aging process.

TABLE 3. Isthmus weight as a percentage of BW and as a percentage of oviduct weight in F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated Single Comb White Leghorn laying hens at 20, 36, 44, 46, 48, and 60 wk of age in Trial 2

Age (wk)	FMG free[1] (% of BW)	FMG inoculated[1] (% of BW)	FMG free[3] (% of oviduct weight)	FMG inoculated[3] (% of oviduct weight)
20	0.4	0.3	11.0	9.8
36	0.4	0.4	8.8	8.7
44	0.4	0.5	12.8	13.4
46	0.6[a]	0.4[b]	15.0[a]	10.6[b]
48	0.4	0.4	11.0	11.1
60	0.4[2]	0.4[2]	11.0[4]	10.9[4]

[a,b]Means within parameter and week of age among treatment groups with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 4, and based on pooled estimate of variance SEM = 0.04 for Weeks 20, 36, 44, 46, and 48.

[2]n = 24, and based on pooled estimate of variance SEM = 0.02 at Week 60.

[3]n = 4, and based on pooled estimate of variance SEM = 0.77 for Weeks 20, 36, 44, 46, and 48.

[4]n = 24, and based on pooled estimate of variance SEM = 0.31 at Week 60.

TABLE 4. Vaginal weight as a percentage of BW and as a percentage of oviduct weight in F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated Single Comb White Leghorn laying hens at 20, 36, 44, 46, 48, and 60 wk of age in Trial 2

Age (wk)	FMG free[1] (% of BW)	FMG inoculated[1] (% of BW)	FMG free[3] (% of oviduct weight)	FMG inoculated[3] (% of oviduct weight)
20	0.3[a]	0.2[b]	9.8[a]	7.0[b]
36	0.3[a]	0.2[b]	5.8[a]	4.3[b]
44	0.2	0.2	4.9	4.4
46	0.2	0.2	5.4	5.2
48	0.2	0.1	5.0	4.0
60	0.2[2]	0.2[2]	5.6[4]	6.2[4]

[a,b]Means within parameter and week of age among treatment groups with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 4, and based on pooled estimate of variance SEM = 0.03 for Weeks 20, 36, 44, 46, and 48.

[2]n = 24, and based on pooled estimate of variance SEM = 0.01 at Week 60.

[3]n = 4, and based on pooled estimate of variance SEM = 0.77 for Weeks 20, 36, 44, 46, and 48.

[4]n = 24, and based on pooled estimate of variance SEM = 0.31 at Week 60.

To develop an egg in a 25-h period, each reproductive tract segment has a task to complete within a certain time frame (Wyburn et al., 1970; Sturkie and Mueller, 1976; Johnson, 2000). However, any alteration in this process could result in reduced rates in egg production. Furthermore, timing of egg production has been shown to be affected by oviductal colonization by MG (Domermuth et al., 1967; Carlson and Howell, 1967; Hitchner et al., 1980). Reductions in relative magnum length due to FMG may also interfere with albumen deposition. Furthermore, as isthmal and vaginal functions control shell deposition and then oviposition, respectively, decreases in relative isthmus weight at 46 wk of age and vaginal weight at both 20 and 36 wk of age may have delayed egg production in FMG-treated birds.

Branton et al. (2002) reported that FLHS incidence was delayed in hens inoculated at 10 wk of age with nonpathogenic *Mycoplasma gallinarum*. However, in the current study, FLHS incidence was increased by approximately 50% in FMG-inoculated birds. Walzem et al. (1999) reported that the liver is primarily responsible for the production of yolk very low density lipoprotein, which is a major yolk precursor. They also reported that inefficient or aging hens appear to lose the ability to correctly assemble yolk very low density lipoprotein, which results in decreased egg production. *Mycoplasma* species may be cultured from the avian liver (Sahu and Olson, 1976), therefore, the livers of birds infected with MG may react similarly to that of inefficient or aging hens. A decrease in liver moisture at 20 wk and an increase at 44 wk of age in treated birds also suggest a decrease in lipid catabolism prelay and an increase in lipid catabolism in the liver of infected birds after peak production. Intestinal characteristics were not influenced by FMG inoculation, and this may be due to the fact that the average temperature of the avian intestinal tract is above 37 C, which is the optimal growth temperature of MG (Razin and Freundt, 1984; Kleven, 1997). Also, mycoplasmas lack a cell wall and are very fragile (Yoder, 1975; Kleven, 1997; Ose et al., 1979; Timms et al., 1989), and a highly acidic intestinal environment may not be adequate for reproduction and growth. In fact, mycoplasmas tend to colonize in more basic (pH = 7.8) environments, such as the upper respiratory and lower reproductive tracts (Hall, 1962; Vardaman, 1967; Kleven and Yoder, 1989). Reductions in feed efficiency in MG-infected birds have been reported (Domermuth et al., 1967; Rodriquez and Kleven, 1980); however, this may, in part, be due to initial colonization of MG in the upper respiratory tract.

An understanding of the pathogenic and physiological processes associated with MG infections may lead to new approaches to the treatment and control of MG. These data demonstrate that alterations in performance and egg characteristics of layers inoculated with FMG at 12 wk of age are related to mutual functional disturbances in the liver, ovary, and oviduct without concomitant intestinal changes.

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